SEQUENTIAL PATTERN OF DECOMPOSITION OF BEECH LITTER WITH SPECIAL REFERENCE TO MICROBIAL SUCCESSION

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THE litter layer has been a locus of intensive study in microbiology since it habours an active saprobic population. The microbial activities in the litter layer are characterized by various transformations of fresh plant debris. There have been many microbiological studies on the so-called litter under the natural condition. Among them, Chesters (1950), Garrett (1956), Webster (1956, 1957), Waid (1957), Pugh (1958), Parkinson and Kendrick (1960), Hudson (1962), Kendrick and Burges (1962), Caldwell (1963) and Hering (1965) initiated an approach to the mechanism of the fungus succession, while Falck (1930), Waksman, Tenny and Stevens (1928), Saitô (1957), Burges (1958) and Carré (1964) emphasized the relationship between chemical changes in the decomposing litter and the participating microflora.

Concerning the fungus flora of decomposing beech litter, Caldwell (1963) made a detailed investigation on the sequence of fungi in the beech stem and root buried in the soil. Carré (1964) studied the changes in the fungal flora and chemical constituents of the decomposing beech cupules.

This paper deals with the successive changes of the breakdown processes of beech leaves and the associated microbial succession, particularly fungus succession. The relationship between litter-inhabiting microorganisms and litter-feeding animals in relation to the decomposition is also discussed.

RELATIONSHIP BETWEEN PROCESS OF DECOMPOSITION AND MICROBIAL POPULATION

The beech (*Fagus crenata*) forest under investigation is a climax forest with the saplings and scattered oak (*Quercus crispula*). The field layer was almost open. The litter layer was naturally preserved. The depth of the deposit reached 2.5 cm to 8.0 cm varying from place to place. The soil was a brown forest soil with little podsolization. In L- and F-layers, there were several kinds of fallen leaves, such as brown leaves of pH 5.1 to 5.3, yellowish leaves, pH 4.0 to 4.8, mouldy leaves, pH 3.7 to 4.0, grayish brown leaves, pH 5.6 to 5.7 and etc. These leaves may well indicate the process and degree of decomposition under the complicated natural environment. The ecological disposition of the main microbial population and the decomposition pattern of the decaying leaves are illustrated in Fig. 1. The summarized

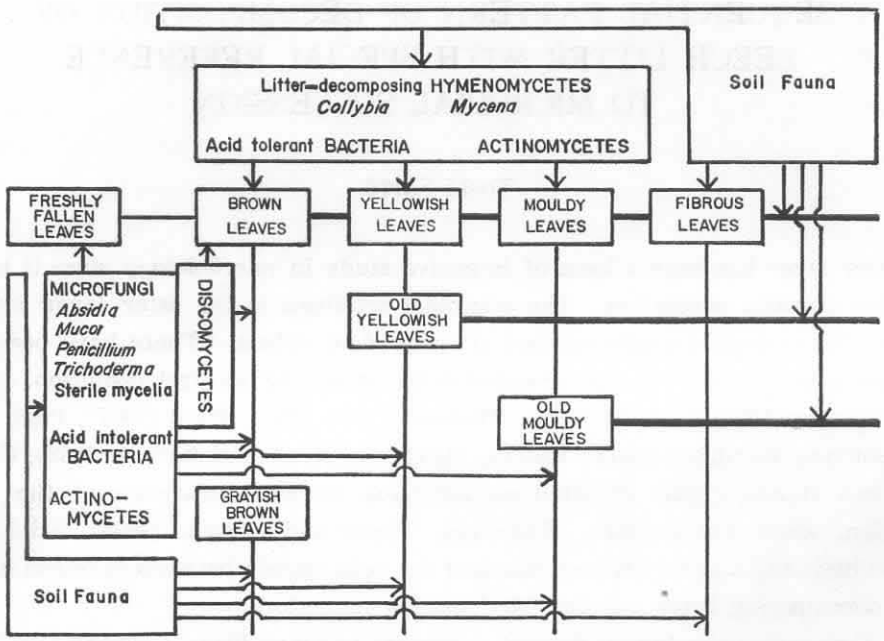

Fig. 1. The sequential pattern of the decomposition of the beech litter in relation to the ecological disposition of the main microbial population.

schema is based on the combination of the chemical and microbiological analyses in both field and laboratory studies.

The fungus population is often considered to represent the predominant group of a microbial community at the early stages of decomposition of forest litter; saprophytic bacterial and actinomycetous populations subsidiary. The same situation was found in this work.

The follicolous fungus flora seemed to be already present on the intact leaf before they reached the surface of the litter layer. In this connection, white and dark sterile mycelia, *Fusarium* sp., *Tripospermum myrti* and *Aureobasidium pullulans* were exclusively isolated from the leaf surface by the use of washing techniques. According to Cooke (1963), *A. pullulans* has the ability of using the simple organic compounds found in nature and of growing in a wide variety of locations. These fungi are sooner or later replaced by the groups possessing higher saprophytic ability.

Among the so-called soil fungi, the first to appear are some of Mucorales, such as *Absidia glauca*, *Mucor ramannianus* and the imperfect fungi, *Penicillia* producing sclerotia, particularly *P. lapidosum* and *P. raistrikii*. These fungi seem to be resistant to the rapid fluctuation of the environmental conditions, such as dryness and exposure, in addition to the advantage of the high germination and growth rate. In the decomposition of the woody material, phycomycetes play a very insignificant

role (Caldwell, 1963).

The above mentioned groups of fungi were followed by discomycete, *Dasyscypha* sp. Small fruit bodies of about 1 mm in height of this fungus were found very frequently on the moistened brown dead leaves. These fruit bodies were seen at 8 to 10 months after the leaf fall. According to Carré (1964) many kinds of the fruiting bodies of discomycetes can be found on the decaying beech cupules throughout the year and these are more affected by weather than by the accumulation and availability of food substrates during the first two years of decay.

As the result of the growth of the first colonizer and the cup fungi, the freshly fallen leaves gradually change their color to dull brown. These attacks, however, are not serious as seen from the coefficient of weight and decomposition (Saitô, 1956) and the loss in weight of the infected leaves in the laboratory culture (Saitô, 1960). In the earlier stages of the leaf decomposition there is only a reduction of water soluble substances, the sugars and the simple compounds in particular.

When these brown leaves are invaded by the late-arriving litter-decomposing hymenomycetes, *Collybia* spp., and *Mycena* spp., a vigorous decomposition occurs resulting in a large loss in weight of the leaves. This pattern is a successive series of leaves from yellowish to mouldy and fibrous as shown in the schema. As already reported (Saitô, 1960, 1965) the chemical changes in this process which presumably correspond to the white rot characterized by the increase in available nutrients and also by the marked fall in acidity. Hence, the hymenomycete-infected leaves, namely the mouldy leaves, become more favorable for acid tolerant bacteria which could not enter the earlier community. In this sense, the microbial population consisting mainly of litter-decomposing hymenomycetes and acid tolerant bacteria seems to play a prominent role in this process with the rapid decomposition rate. Because of the development of the subsequent bacterial population, the hymenomycetous mycelia and mycelial strand gradually become feeble. Saitô (1965) pointed out that there was a close interrelation between the cessation of hymenomycetous growth and the appearance of the subordinate microfungi. Accordingly, the old, yellowish, mouldy leaves that have been free from the active mycelia or mycelial strand, serve as an excellent substratum for the secondary growth of microfungi competing with one another for the available nutrients. It is of interest to note the abundant occurrence of yeasts such as *Candida* and *Cryptococcus* only on these leaves (Saitô, 1956; Kuraishi, 1958). The serial development of the microbial population on these old leaves may be regarded as of the secondary succession.

There is another pattern of decomposition with a slow rate from brown leaves to grayish brown, which is not the result of the hymenomycetous attack. In this pathway, cellulose is gradually decomposed, while lignin is largely left intact. Unlike those which can be easily isolated by the routine plate method, the kind of

microbial community that actually contribute to this decomposition process is still not clear and cannot be studied satisfactorily by the available methods (Warcup, 1955, 1959). Microscopic observation revealed that the brownish sterile hyphae is widespread usually on the grayish brown leaves. Unfortunately, the hyphae failed to develop on the various culture media. The subsequent bacterial population in this process differs from that of the white rot process and is not found in a large number. The majority of them are acid intolerant bacteria. In addition, in the later stages of this decomposition process, a relatively large number of actionomycetes appear. Whether the increase in number was due to their ability of being capable of utilizing less decomposable components or to merely the lack of competition is still not clear.

The constituents of the grayish brown leaves are subject to decomposition, which is similar to the brown rot in wood decay. Throughout this study, however, no evidence was found to indicate that the wood-rotting hymenomycetes took part in the decomposition of the leaf-litter. This result agrees with that of Lindeberg (1962) who contradicted the *Coniophora* type-decomposition reported by Falck (1939). Thus, the decomposition process can be divided into two systems, though these two kinds of process are not necessarily separated from one another in the natural habitats. The development of each process is often modified by the changes in the environmental factors. For example, the secondary colonization of microfungi on the yellowish and mouldy leaves free from active hymenomycetous mycelia lead again to the brown rot process. In the culture condition, it was found that when a micromycete and a hymenomycete were grown together on beech leaves, the latter became predominant covering the former which had spread at first (Saitô, 1960). In this case, if the micromycetes were associated with bacteria they showed a marked inhibition against the invasion of the hymenomycete. In the natural community, strictly speaking, it would be impossible to determine the coaction of fungi apart from the association of bacteria.

Further microbiological breakdown in both decomposition processes tend to somewhat arrest, while the decomposing leaves, even in the white rot process, still retain their structure to a considerable extent. Then, the microbiological decomposition seems to be followed by the feeding of the litter-inhabiting fauna.

This study was carried out in a single locality, but the microflora might be different in different habitats. Webster (1956, 1957) described a basic fungus pattern as decomposition proceeded in cocksfoot tussocks in three localities. It should be borne in mind, however, that distinct differences with regard to the fungal pattern in the adjacent parts of the substratum have been observed by Tribe (1960), Caldwell (1963) and Carré (1964).

Interaction Between Microflora and Soil Fauna

In addition to the microbiological decomposition of the litter, it is very important to study the coactions and the joint process between litter-inhabiting microorganisms and litter-feeding animals, as already pointed out by Birch and Clark (1953), Murphy (1955), Kubiena (1955) and Kononova (1961).

In this beech litter layer, various kinds of soil animals were observed, such as springtails, insect larvae, myriapods, mites in common and earthworms rarely. Among these animals, mites and springtails were exceedingly abundant. They are known to be the most important groups in the acidic forest litter. As to the microfaunal population in the beech litter, Stout (1962) reported that rotifers and copepods besides the protozoa are abundant.

Animal feeding of the fallen leaves was conspicuous in the mouldy leaves, particularly old ones and to a less extent in the old yellowish leaves. Masses of faeces were found under the hymenomycete-infected leaves in the litter layer. This outstanding feature may indicate a chemical change in the decomposing leaves as well as the mechanical comminution of the leaves. On the contrary, the brown and grayish brown leaves are likely to be unfavorable for the food of these animals. The litter-feeding animals seem to show a marked preference for the leaves infected with litter-decomposing hymenomycetes in comparison with those of the brown rot process. This preference was confirmed by the following experiment in the laboratory. Larvae of the dipterous fly (*Scatopsidae*) were bred on each of the above mentioned fallen leaves in Petri dishes. The mouldy leaves were eaten by the larvae most rapidy, and transfered into faeces, while the brown and grayish brown leaves remained almost intact. It seems likely that the preference for fallen leaves is not merely due to species of tree (Murphy, 1955), but to the pattern and its degree of decomposition. Eventually, hymenomycetes convert much of the leaves into their own mycelia that are the form on which mites and springtails can feed. From the fact mentioned above, it seems probable that one of the roles of litter-inhabiting microorganisms is to prepare the infected litter for utilization by other animals.

An Approach to the Mechanism of Fungus Succession

Garrett (1956) established the concept of microbial succession with special reference to fungus sequences, based on their substrate relationships and competitive saprophytic colonization. He assumed that the autogenic succession of heterotrophic microorganisms progressively brought about the depletion and exhaustion of substrates in contrast to the improvement of habitat in that of a green plant community, and eventually the final stage of microbial succession becomes zero.

In general, the phycomycetes tend to come first when fresh plant residues

are added, they are followed by ascomycetes and fungi imperfecti, and finally by basidiomycetes. This sequence of fungi is well known, particularly on the dung of herbivorous animals and compost, and has been accepted as a model to explain other mycoseres. In the present study, similar successive waves of fungus population were observed, although less remarkable as compared with those on the dung and nutritive plant materials. The fungus species to occupy the beech litter in the early stages is stenophagous that is gradually substituted by omnivorous fungi which possess greater saprophytic ability. It is very likely, however, that there are various mycoseres due to the variety of the substrata and of the environments. For instance, Caldwell (1963) has elucidated the existence of a fungus succession in the beech twigs in soil; although there are qualitative and quantitative differences between wood and bark, *Cylindrocarpon didymum* is the dominant fugus and is replaced by *Trichocladium opacum* in the later stages. Besides, *Hypoxylon*, *Penicillium*, *Trichoderma* and others are the most frequent fungi next to *Cylindrocarpon* spp. The study by Hering (1965) has shown that the microflora occurring on the leaves of oak, birch, hazel and ash are composed characteristically of pyrenomycetes and imperfect fungi up to one year after leaf fall, but subsequently are replaced by a common flora dominated by *Penicillium* and *Trichoderma*. Besides, there is an interesting discussion by Kreutzer (1965) in relation to the microbial succession in treated soil. Here also, there might be some problems to add to the general sequences mentioned above.

In the laboratory culture, most of cellulose-decomposing fungi such as *Trichoderma viride* and *Penicillium lapidosum*, failed to develop on the infected beech leaf-powder which had already been subjected to fully mucoraceous attacks (unpublished data). The reverse is true for Mucorales such as *Absidia glauca* and *Mucor ramannianus*. It seems unlikely that imperfect fungi and ascomycete begin to invade after the flare-up of Mucorales. Probably, these have already occupied some food bases in the relatively earlier stages with some of sugar fungi. It is noticeable that these fungi depend largely upon sugar and simple carbon compounds, though some of them decompose cellulose to some extent. In other words, sugar fungi and cellulose decomposer (a group incapable of digesting lignin) are relatively close groups both in time and in space. Their colonizations overlap partially in the same niche. Sugar fungi are considered to be quite a competent pioneer on the plant debris besides endophyte (Waid, 1957) and follicolous fungi (Parkinson and Kendrick, 1960; Kendrick and Burges, 1962). According to Tribe (1962) however, *Pythium oligandrum* can be classified as a sugar fungi in a nutritional sense, but its ecological status needs re-assessing as it grows on pure cellulose with cellulolytic fungi in soil. Moreover, Harper and Webster (1964) described that there was no clear correlation between the latent period for spore germination and germ-tube growth rate, and observed the time of appearance of fruit bodies from the result of the experimental analysis

of the coprophilous fungus succession. These facts might explain the reason why some fungi are isolated freely through the course of decomposition in contrast to fungi which occupy a fairly well-defined place in the succession. In the case of the fungal sequences mentioned above, the pre-treatment by the earlier colonizers, i. e. the reaction, is not necessarily essential to the development of the late-arrivals.

On the other hand, lignin decomposer such as *Collybia* spp. and *Mycena* spp. can colonize on the micromycete-infected leaves (Saitô, 1960). Because of the striking feature of utilizing the membranous materials, a sharp line can be drawn between the hymenomycetes and the ealier groups described above.

So far as the white rot process is concerned, the microfungi can grow depending on the accumulation of the available nutrients after the vigorous development of the litter-decomposing hymenomycetes (Saitô, 1965). Under the field condition, the recolonization and new invasion of microfungi are much less and more irregular than those in the laboratory. This discrepancy is probably due to the complicated competition among various kinds of microorganisms, as well as the inadequate temperature, humidity and water content in the natural habitats. It is of interest that the litter-decomposing hymenomycetes tend to improve the nutritional condition in terms of the substrates to support the growth of many kinds of microorganisms, though there is a decline of energy as the decay occurs. This is the case in which late-arriving species require the reaction of the early colonist.

Similar situations are also seen in Tribe's work (1962) mentioned above. The chemical energy of fallen leaves, of course, is at the maximum at the beginning. Nevertheless, the freshly fallen leaves are not always considered to be excellent habitats. Burges (1958) pointed out from the results of the microbial sequences on pine needles that there was an improvement of the habitats, as the substratum became soft and had higher water-holding capacity with the progress of decomposition. Furthermore, the same situation can be considered for the growth factors produced during the decomposition. Whether the freshly fallen leaves form excellent substratum may depend on the surface structure and chemical natures of the leaf itself and the water condition in the habitats. On the other hand, the brown rot process lead to a depletion and exhaustion of the decaying beech leaves.

Although antibiotic production has been demonstrated in the majority of the litter-decomposing hymenomycetes *in vitro* (Saitô, 1958, 1965), no appreciable antibiotic activity was detected in the leaves fallen on the ground. The saprophytic ability and the organic acid production that results in the precipitation of crystals of calcium oxalate around their mycelia and strand, are much more important even in the immediate vicinity of the substrate than the mere production of the antibiotics.

Concerning a microbial succession as compared with that of the green plant, Garrett (1956) pointed out that there is a close analogy between them in the autogenic

succession. In the development of the microbial community, the competition for substrates is of prime importance, just as sunlight is for the green plant. It should be pointed out, however, that the microbial succession differs radically from that of the green plant according to their inherent physiological properties. For example, the requirement of sunlight by the green plant in a community is not quality but intensity in the ecological sense. For convenience, many species are divided into two groups; shade tolerant and shade intolerant. On the contrary, the substrate of microorganisms, so far as the naturally occurring plant debris are concerned, offer the heterogenous complicated media composed of many kinds of substances for heterotrophants consisting of various nutrient types. In the process of autogenic successional changes of the green plant community, changes in light conditions that are brought about by the development of the community, associated with changes in edaphic factor, lead to the appearance of new colonizers with different amplitudes. In the microbial community, however, even if the new competitors do not crowd out the earlier colonizers, the latter will disappear sooner or later when the given available foods are exhausted, unless new substrates are added. It seems likely, therefore, that the appearance of a new competitor is not the prime cause which crowd out the previous occupants.

In the open system in nature, a continuous change can also be brought about by the acting external factors which are constantly altering the microhabitats, such as the addition of fallen leaves, changes in vegetation and the leaching of soluble nutrients from the surface layers. These conditions would lead to the occurrence of an allogenic succession of the microbial world in parallel with the autogenic succession. Although the distinction between the two types of succession should be made clear, it can be assumed that these two types of succession are related closely in a dynamic fashion expressing either successive or irregular transformation from the less-decomposed surface litter to the underlying structureless mass. In this connection, Burges (1963) who referrd to Watt's co-ordinated unit in higher plant ecology, presumed that there are series of up-grade and down-grade steps in the soil also.

As might be expected, a zymogenous microbial community is replaced by an autochthonous community which occupy the majority of the microbial climax community. If the addition of plant debris were maintained indefinitely, the climax would be correspondingly maintained. Then, the ecosystem in the soil would become mature.

SUMMARY

1. In the leaf-litter layer of the climax beech wood, the decomposition process can be divided into two sequential patterns; the white rot process and the brown rot process. They are not always separated in nature, but form a linked process.

2. In the fungus succession in the white rot process, the litter-decomposing hymenomycetes are subsequently replaced by the microfungi in various degrees.
3. In the brown rot process, sugar fungi and cellulose-decomposing fungi are relatively close groups both in time and space, as compared with the hymenomycetes.
4. Litter-feeding fauna showed a remarkable preference for the white-rotting leaves and no preference for the brown-rotting leaves.
5. The pattern of fungus succession was compared with that of the green plant.

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